Subterranean Biology 16: 37–46 (2015)
doi: 10.3897/subtbiol.16.8609
http://subtbiol.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Subterranean Biology
Published by The International Society for Subterranean Biology

Food sources of selected terrestrial cave arthropods

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Academic editor: *O. Moldovan* | Received 17 September 2014 | Accepted 18 June 2015 | Published 8 July 2015

http://zoobank.org/D1EFD2A4-3B3B-45FC-B91F-EC1EB0398392

Citation: Smrž J, Kováč L, Mikeš J, Šustr V, Lukešová A, Tajovský K, Nováková A, Režňáková P (2015) Food sources of selected terrestrial cave arthropods. Subterranean Biology 16: 37–46. doi: 10.3897/subtbiol.16.8609

Abstract

As caves represent an extreme biotope with limited food sources, one might expect cave animal communities to exhibit low feeding specialization and to consume generally whatever organic matter is available. To test this hypothesis, we studied the feeding habits of several arthropod species in Slovakian and Romanian caves. A microanatomical approach utilizing histological methods was selected for this study. While saprophagous animals dominated, our study revealed variability within this nutritional group. Preferences ranged from fungal propagules (for the millipede *Trachysphaera costata*) to bacteria on bat guano (for the oribatid mite *Pantelozetes cavaticus*) and to cyanobacteria (for the microwhip scorpion *Eukoenenia spelaea*). The terrestrial isopod *Mesoniscus graniger* consumed a mixture of organic and inorganic substrates with plant material in various caves. These findings confirm an adaptability and phenoplasticity and, hence, a variability of characteristics developing under the pressure of extreme environmental factors.

Keywords

Caves, feeding habits, Isopoda, Diplopoda, Collembola, Acari

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Introduction

Caves constitute an extreme or at least unique biotope in terms of their abiotic as well as biotic conditions (Seckbach 1999; Culver and Pipan 2009). Abiotically, they differ from epigean habitats by their relatively low and stable temperature, poor or no light, and mostly high moisture (Vandel 1965). These conditions affect biotic factors such as food availability for the cave biota. Saprophagous, necrophagous, carnivorous and microorganism eaters (bacteriophagous, mycophagous and algivorous) can be all found in such an environment but their feeding habits have rarely been thoroughly studied. The diet of terrestrial cave arthropods has been predominantly studied in crickets (e.g. Lavoie et al. 2007; Di Russo et al. 2014) and beetles (e.g. Paoletti et al. 2011, 2013; Bradford et al. 2014). However, very limited data are available regarding the feeding habits of other cave arthropods, such as Acari, Isopoda, Diplopoda and Collembola.

We hypothesized that impoverishment of food sources in the cave environment results in poor consumer communities and probably in reduced specialization of nutritional niches. We should have expected, therefore, to find general consumption of whatever food is offered and without any conspicuous selection, as well as grazing of all available organic matter regardless of its palatability or digestibility. To test this hypothesis, we sampled representatives from several invertebrate groups in selected Slovakian and Romanian caves and then analyzed their gut content.

Material and methods

Nearly all animals were sampled individually in Ardovská Cave (south-eastern Slovakia), and only *Mesoniscus graniger* (Frivaldszky, 1865) (Crustacea, Isopoda, Oniscidea) originated from Domica Cave (south-eastern Slovakia) and three Romanian caves (Zidită, Măgura, Fânaţe). The research adhered to the conditions of Licence # 3102/2009- 2.1/jam, from the Ministry of the Environment of the Slovak Republic, certificate of competency per Act No. 543/2002, and for Romania the authorization no. 340 of the Speleological Heritage Commission of the Ministry of Environment, Waters and Forests.

In details, representatives of several animal groups were collected for analysis of gut content:

– from Ardovská Cave:

16 specimens of microwhip scorpion *Eukoenenia spelaea* (Peyerimhoff, 1902) (Arachnida, Palpigradi);

12 specimens of oribatid mites *Pantelozetes cavaticus* (Kunst, 1962) (Acari, Oribatida);

10 specimens of millipedes *Trachysphaera costata* (Waga, 1857) (Myriapoda, Diplopoda);

22 specimens of springtails, 10 of *Protaphorura armata* (Tullberg, 1869). and 12 of *Folsomia candida* Willem, 1902 (Hexapoda, Collembola).

– from Domica Cave and Romanian caves (Zidită, Măgura, Fânaţe):
27 specimens of terrestrial isopods *Mesoniscus graniger* (Slovakia – 10 individuals, Romania – 17 individuals).

A microanatomical approach utilizing histological methods was selected for this study (Smrž 2002). Animals were fixed in modified Bouin-DuBosque-Brasil fluid (Smrž 1989) then embedded in Paraplast Plus (Fluka), sectioned on a Leica 2155 rotation microtome (0.005 mm thickness), and stained in Masson's trichrome. Occasionally, this was combined with observation using a Nomarski differential interference contrast prism (DIC). All sections were examined under an Olympus AX-70 microscope. The type and amount of gut contents (presence of food in all parts of gut or only in some ones), activity of the gut walls, and storage of nutrients (glycogen) inside the body were observed, according to the method described by Smrž (2013).

Fresh specimens of *Eukoenenia spelaea* were viewed using a Leica TCS SP5 X confocal microscope with autofluorescence and red channel (emission spectrum 588–683 nm, excitation wavelengths 548–557–567 nm).

Plating of cave substrate on the cyanobacterial medium BBM in 1.7% agar plates was performed to confirm the cyanobacteria presence in Ardovská Cave.

Results

The results of gut contents in all tested species were the same inside each taxon.

Spherical cells (mostly 0.003–0.008 mm or larger) in the guts of palpigrades (*Eukoenenia spelaea*) were determined to be cyanobacteria (Fig. 1). Under confocal microscopy, palpigrade guts (4 specimens) were observed to be filled or crowded by spherical cells (Fig. 2). The digestibility of those cells was confirmed by very intensive deposition of glycogen in the gut diverticula. All sectioned microwhip scorpions (12 specimens) exhibited the same characteristics. The plating of cyanobacteria from cave substrate confirmed the presence of those cyanobacteria in Ardovská Cave. Unlike the cyanobacteria that had been swallowed, those plated had glycocalyx (Fig. 1, blue arrowhead).

Pantelozetes cavaticus mites, meanwhile, were characterized by a food bolus in the mesenteron, with several concentric membranes and many small bacterial cells between them (Fig. 3). Several specimens exhibited some particles of amorphous red mass in the bolus. The palatability of grazed food was linked to glycogen particles around the gut and a very intensive apocrine secretion in the blind projection of the mesenteron (the mesenteric caeca).

On the other hand, organic particles, and especially microfungal conidia, were grazed by the robust millipede *Trachysphaera costata* (Fig. 4). Those were mixed with wood fragments within an organic amorphous mass.

The observed springtails can be differentiated into two nutritional groups. In specimens of *Protaphorura armata* (Onychiuridae) the mesenteron contained a uniform mass of bacterial cells (Fig. 5), while in those of *Folsomia candida* (Isotomidae) a mixture of

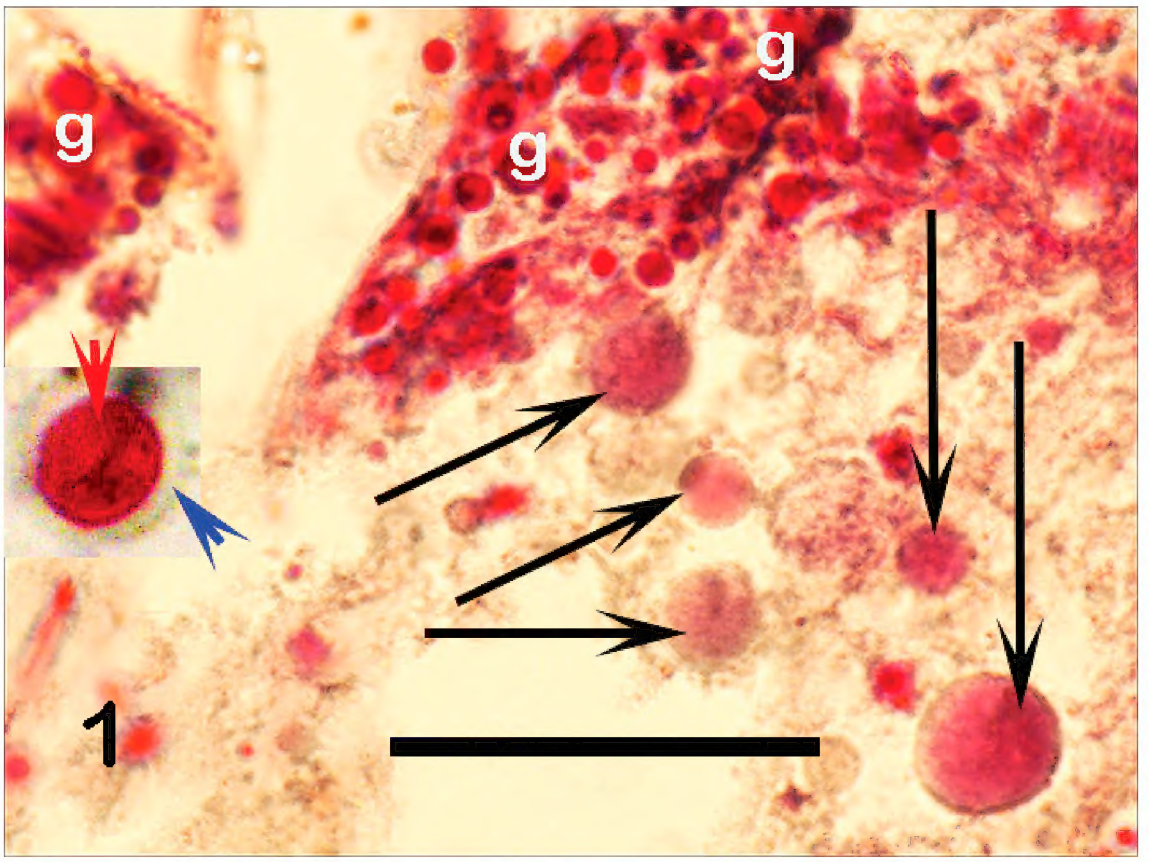

Figure 1. *Eukoenenia spelaea* – gut with food (large cells, indicated by black arrows). Red arrowhead = plated cyanobacteria in cave substrate (inset), blue arrowheads = glycocalyx. Stained with Masson's trichrome. Abbreviation: **g** glycogen deposits. Scale bar: 0.02 mm.

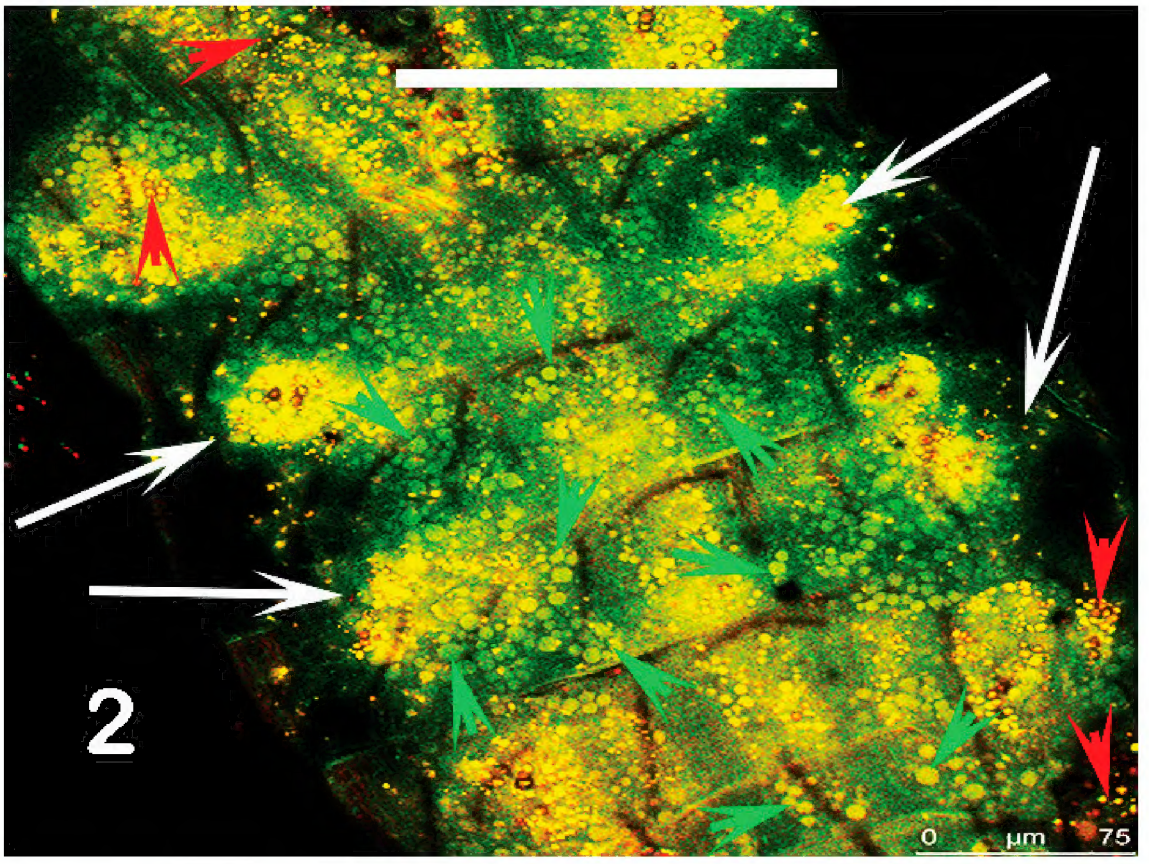

Figure 2. *Eukoenenia spelaea* – gut, confocal microscopy. Autofluorescence under red channel (emission 588–683 nm, excitation 548–557–567 nm). White arrows = gut diverticula, green arrowheads = cyanobacteria, red arrowheads = glycogen deposits. Scale bar: 0.1 mm.

Figure 3. *Pantelozetes cavaticus* – mesenteron with concentric bolus. Black arrowheads = apocrine secretion of the walls of mesenteron, red arrowhead = particle of amorphous mass in food bolus. Stained with Masson's trichrome. Abbreviations: **ba** bacteria loosely out of food bolus **fb** food bolus **g** glycogen deposits **go** gonads **me** cavity of mesenteron. Scale bar: 0.02 mm.

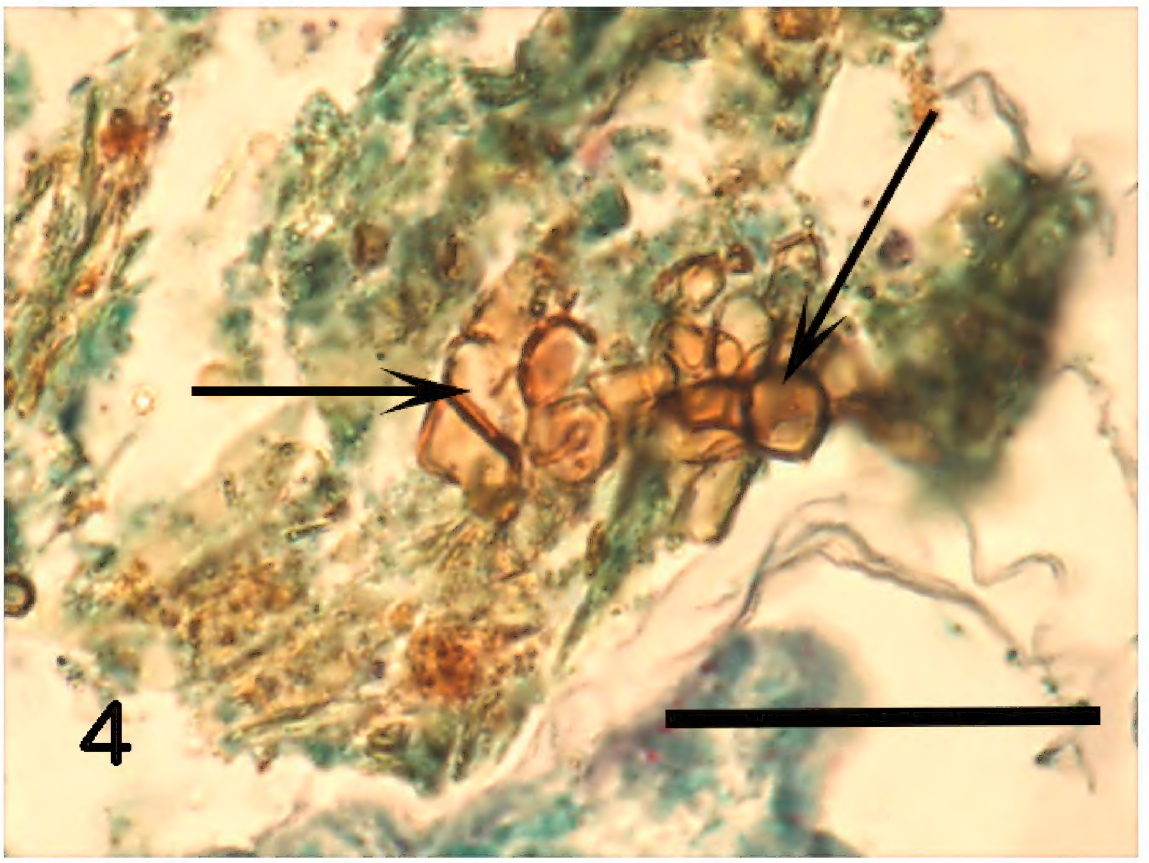

Figure 4. *Trachysphaera costata* – gut with microfungal conidia (arrows). Stained with Masson's trichrome. Scale bar: 0.02 mm.

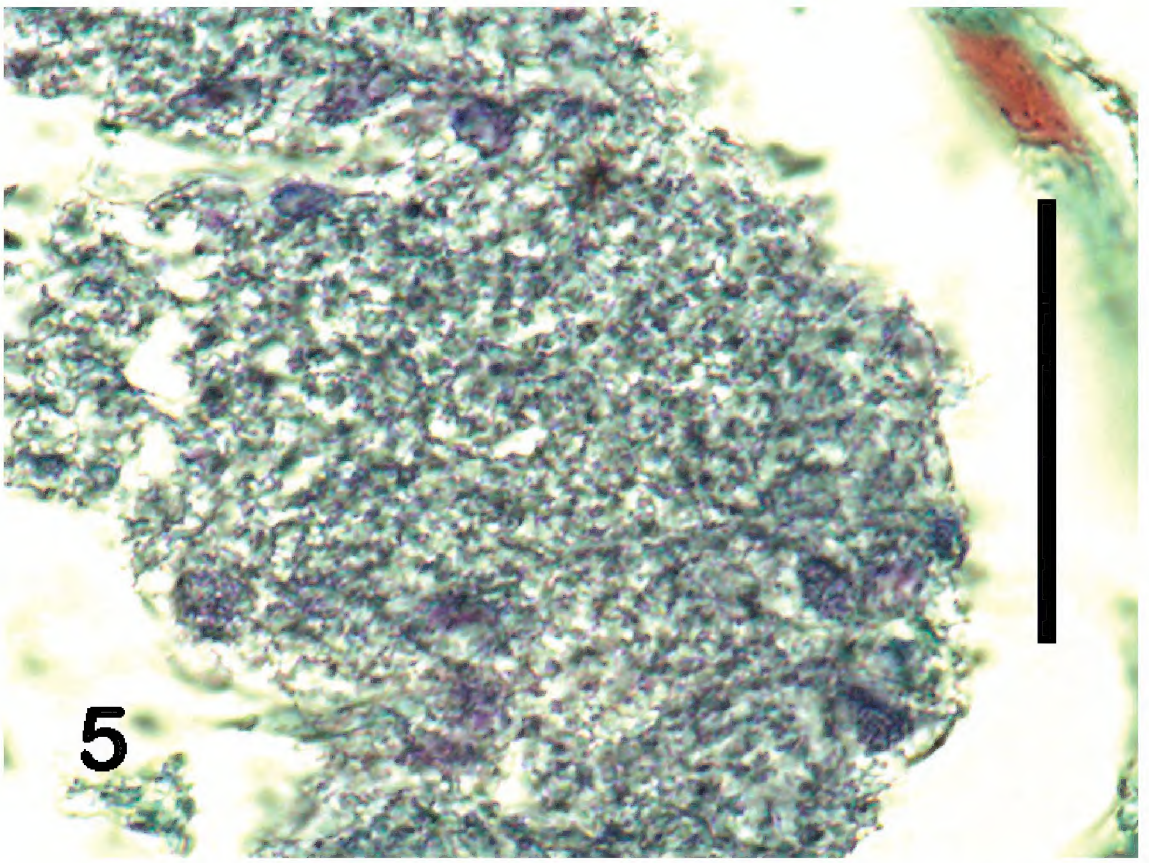

Figure 5. *Protaphorura armata* – gut with bacterial cluster. Stained with Masson's trichrome. Scale bar: 0.02 mm.

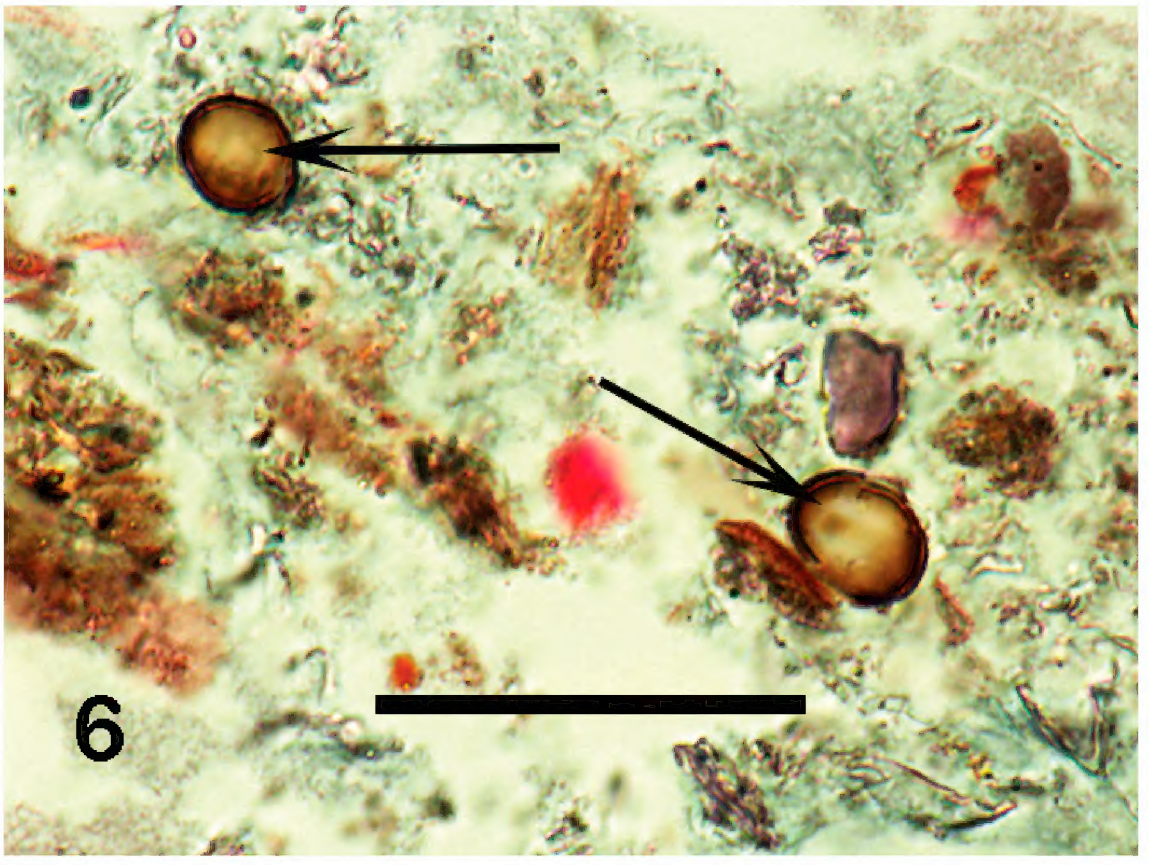

Figure 6. *Folsomia candida* – gut with microfungal conidia (arrows). Stained with Masson's trichrome. Scale bar: 0.02 mm

organic particles including microfungal conidia were found (Fig. 6). The digestibility of this substrate was confirmed by intensive apocrine secretion of the gut walls.

The terrestrial isopod *Mesoniscus graniger* was sampled in several Carpathian caves. We were thus able to compare the gut contents of individuals from distant localities.

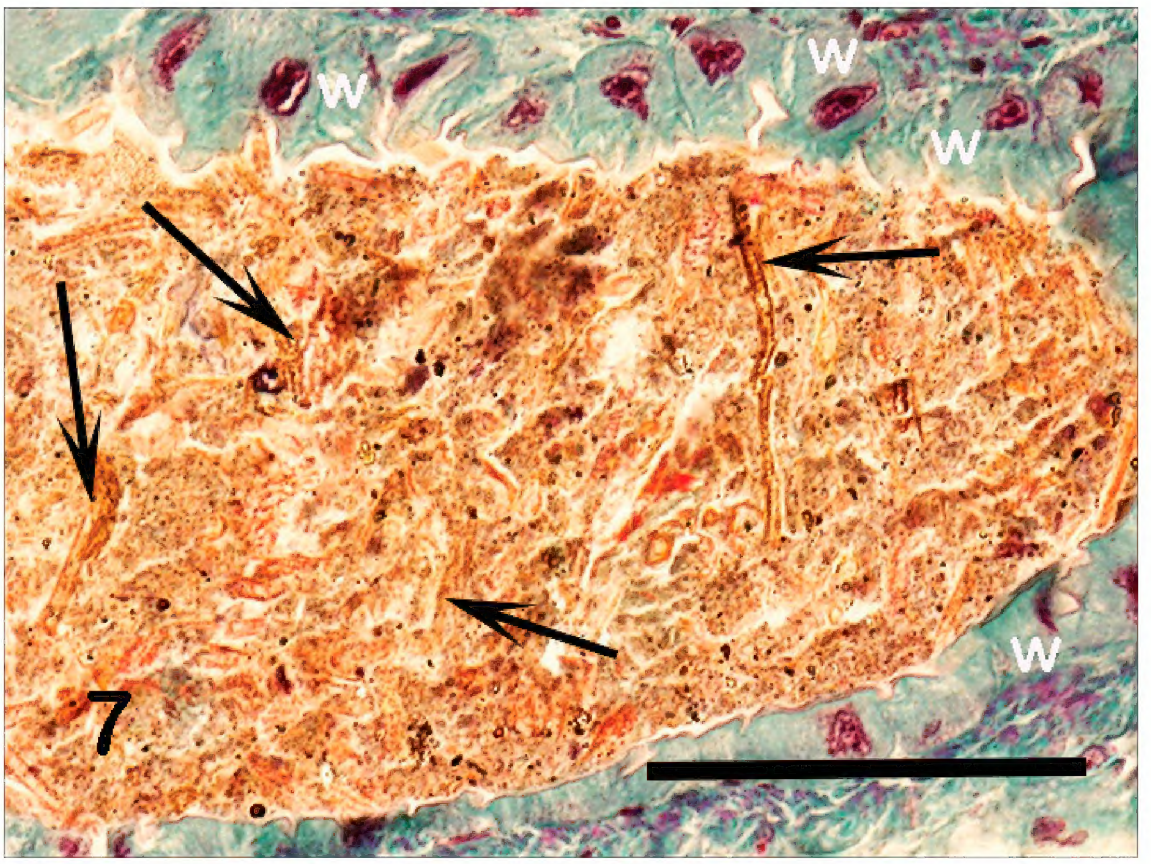

Figure 7. *Mesoniscus graniger*, Domica Cave, Slovakia – gut with food (plant remnants indicated by arrows). Stained with Masson's trichrome. Abbreviation: **w** gut walls with red nuclei. Scale bar: 0.05 mm.

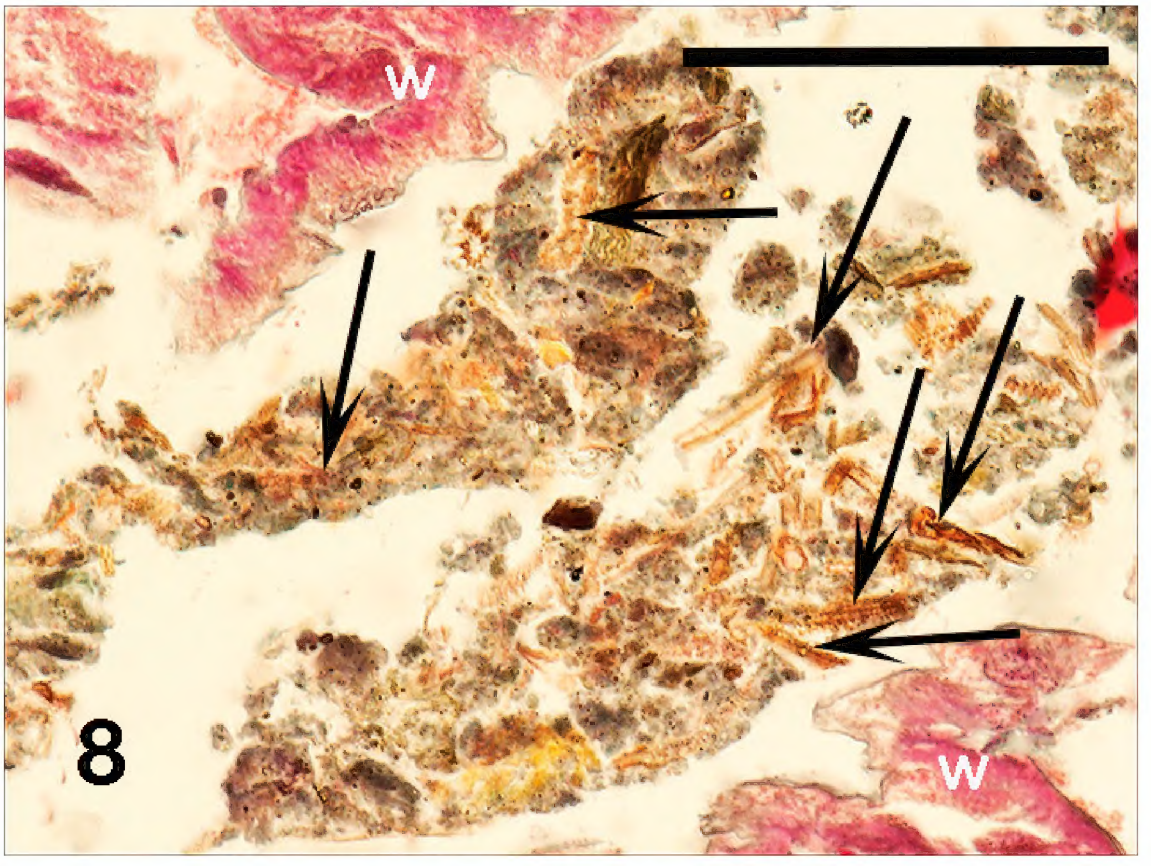

Figure 8. *Mesoniscus graniger*, Zidită Cave, Romania – gut with food (plant remnants indicated by arrows). Stained with Masson's trichrome. Scale bar: 0.05 mm.

All were similar to one another regardless of the cave. The dominating mass was comprised of a mixture of organic and inorganic substrates with remnants of plants, both having no affinity for the used stain (Figs 7, 8). For the overwhelming majority of the analyzed animals, no fungal propagules were found.

Discussion

In spite of the limited food sources for cave-dwelling animal species, some nutritional specialization is evident. Food sources are used very thoroughly. First details of prey capture and cyanobacteria diet in palpigrades were published by Smrž et al. (2013). Cyanobacteria known for its ecological plasticity, with resistance against low or high temperatures, dryness, as well as poor light (Fay 1965) is able to grow also in caves.

The oribatid mite *Pantelozetes cavaticus* inhabited heaps of bat guano. The specimens in this study exhibited a concentric structure of food bolus in their mesentera. Such structure with bacterial cells between membranes confirms bacterial nutrition (Smrž 1989). Bacteria are a rather palatable and digestible food, as confirmed by glycogen deposition around the gut. The amorphous red mass in the bolus was clearly guano. The millipede *Trachysphaera costata* exhibited frequent consumption of fungi. Bacterial nutrition was demonstrated in the onychiurid springtails *Protaphorura armata*, while a selection of microfungal conidia were found in the gut of the isotomid *Folsomia candida* as confirmed by secretion activities in their gut walls. The food selection of the Collembola appears to be broad, reflecting various microhabitats inhabited by representatives of this group (Tebbe et al. 2006). *F. candida* and *Heteromurus nitidus*, frequent cave inhabitants, may even feed on nematodes (Lee and Widden 1996; Fiera 2014).

The isopod *Mesoniscus graniger* represents a contrasting case in nutrition selection, as seen in its low specificity of grazed food. Its gut was filled with mineral and organic material, with no indication of selection. Such condition was recorded in Domica Cave in Slovakia as well as in caves in Romania. On the other hand, that might be indicative of some consistency or even some type of selection in the feeding habit of this species. A wide range of food (fungi, algae, bat guano) was recorded in feeding experiments in Domica Cave, Slovakia (Šustr et al. 2005). However, a subsequently improved methodology that included more detailed laboratory feeding preference tests showed that *M. graniger* exhibits a clear preference for grazing on cave sediment and on laboratory cultures of some algae species over other species of cave algae and fungi (Nováková et al. 2005; Šustr et al. 2005). Both subspecies *M.graniger dragani* (Magura cave) and *M.graniger graniger* (Zidita and Fanate caves) had the same food in their guts. Nevertheless, more details can be found in small populations according to their different mouthparts and also provide more details of the food preferences (Giurginca, Šustr and Tajovský 2012).

While our hypothesis had assumed negligible selection, there is in fact conspicuous food selection by terrestrial arthropods in the studied cave biotopes. The limited food offer seems to be used very unambiguously and thoroughly by the invertebrate communities. Therefore, the competition for food can be actually regarded as very low.

Acknowledgments

The study was supported by a grant from the Slovak Scientific Grant Agency VEGA No. 1/0139/09. The research was funded also by the Academy of Sciences of the Czech

Republic, Research Plan No. AV0Z60660521. The authors thank the Slovak Caves Administration and O. Moldovan and T. Brad (Emil Racovita Institute of Speleology, Cluj-Napoca, Romania) for enabling us to sample cave materials. We thank Gale A. Kirking (English Editorial Services, Brno) for his kind linguistic and stylistic revision and the anonymous reviewers for suggestions and corrections.

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